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Determination of Fluoroquinolone Antibacterial Agents in Sewage Sludge and Sludge-Treated Soil Using Accelerated Solvent Extraction Followed by Solid-Phase Extraction

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A method for the quantitative determination of human-use fluoroquinolone antibacterial agents (FQs) ciprofloxacin and norfloxacin in sewage sludge and sludge-treated soil samples was developed. The accelerated solvent extraction was optimized with regard to solvents and operational parameters, such as temperature, pressure, and extraction time. A 50 mM aqueous phosphoric acid/acetonitrile mixture (1:1) was found to be optimum in combination with an extraction temperature of 100 °C at 100 bar, during 60 and 90 min for sewage sludge and sludge-treated soil samples, respectively. A cleanup step using solid-phase extraction substantially improved the selectivity of the method. Overall recovery rates for FQs ranged from 82 to 94% for sewage sludge and from 75 to 92% for sludge-treated soil, with relative standard deviations between 8 and 11%. Limits of quantification were 0.45 and 0.18 mg/kg of dry matter for sewage sludge and sludge-treated soils, respectively. The presented method was successfully applied to untreated and anaerobically digested sewage sludges and sludge-treated soils. Ciprofloxacin and norfloxacin were determined in sewage sludges from several wastewater treatment plants with concentrations ranging from 1.40 to 2.42 mg/kg of dry matter. Therefore, contrary to what may be expected for human-use pharmaceuticals, FQs may reach the terrestrial environment as indicated by the occurrence of FQs in topsoil samples from experimental fields, to which sewage sludge had been applied.

Pharmaceuticals can enter the terrestrial environment either by direct disposal of sewage sludge and liquid manure to soils or indirectly through animal medication in fish farming, where medicated feed pellets and feces can end up in sediments. Until now, the terrestrial environment has been studied regarding the environmental fate of animal-use pharmaceuticals because of their input into soils[1] and sediments.[2−4] For human-use pharmaceuticals, however, research has tended to follow their occurrence and fate in the aquatic environment,[5−7] whereas terrestrial exposure is considered a minor route of pollution.[8,9] This prioritization of the aquatic environment for human-use pharmaceuticals is a consequence of the polar nature of the majority of pharmaceuticals and metabolites, which have found their way to the environment via discharge of wastewater effluents into surface waters.[5,6]

Fluoroquinolones (FQs) are highly useful antibacterial agents, particularly because of their broad activity spectrum and good oral absorption.[10,11] They are applied in both human and veterinary medicine, and almost identical structures are used for humans and animals.[12,13] In Europe and in the United States, FQs were introduced for human use in the mid-1980s and approved for therapeutic treatment of livestock in the mid-1990s. In Switzerland, FQs are being primarily applied for treating human infectious diseases (∼4 t/year), whereas in veterinary medicine their use is minor (∼0.5 t/year).[14] Thus, the primary entry route of FQs into the Swiss environment is via human excretion in sewage, as confirmed by the occurrence of the leading human-use FQs ciprofloxacin and norfloxacin in wastewater effluents and surface

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waters.[15] During municipal wastewater treatment, FQs are significantly eliminated from the water stream (79−87%),[15] and their fate is likely to be associated with sewage sludge because of their strong sorption properties.[16−18] Application of sewage sludge to soils may therefore be a potential route for these human pharmaceuticals to enter the terrestrial environment.

Until now, analytical methods for the quantitative determination of FQs in the environment were only available for aqueous samples.[19] Various methods have been described for the extraction of the structurally related quinolone oxolinic acid from marine water and freshwater sediments.[4,20,21] However, the relatively low recoveries[20] and precision[4] obtained with some of the analytical methods spurred the development of new, robust, and powerful methods, which are applicable to a wide range of solid environmental matrixes. Accelerated solvent extraction[22] (ASE) was selected for this study, because it has proven to be successful for the extraction of various organic pollutants in several solid environmental sample matrixes.[23−25]

This paper describes an extraction and cleanup procedure for FQs in sewage sludges and sludge-treated soils using ASE followed by solid-phase extraction (SPE), which in combination with a previously described separation and detection method (LC-fluorescence detection)[19] enables selective, reliable, and quantitative determinations. Ciprofloxacin and norfloxacin were chosen as the specific FQ analytes, since they are the most prescribed FQs in Switzerland and they have already been detected in the aquatic environment here.[15] To the best of our knowledge, this article is the first to report on the occurrence of human-use pharmaceuticals in sewage sludges and in sludge-treated soils.

EXPERIMENTAL SECTION

Chemicals and Materials. Ciprofloxacin (CIP) was obtained from Bayer AG (Wuppertal, Germany), norfloxacin (NOR) from Sigma-Aldrich, and tosufloxacin (TOS) from Abbott Laboratories (Baar, Switzerland). Tosufloxacin was used as a surrogate standard, of which any human use is restricted to Japan.[26] More details on standard solution preparation are given in ref 19. Mixed-phase cation exchange disk cartridges (MPC, octyl phase and benzenesulfonate mixture, high-density 12-μm particle size) were supplied by Varian International AG (Basel, Switzerland). All solvents were of reagent grade or higher quality. HPLC-grade water, acetonitrile, methanol, and 2-propanol were purchased from Scharlau (Barcelona, Spain). Orthophosphoric acid (*o*-H_3PO_4, 85%) and hydrochloric acid (32%) were supplied by Merck (Dietikon, Switzerland). Sodium hydroxide and sodium chloride were purchased from Fluka Chemie AG (Buchs, Switzerland).

Sample Collection. *Sewage Sludge.* Two anaerobically digested sewage sludges and two untreated raw sludges were collected from mechanical−biological wastewater treatment plants near Zurich, Switzerland. The sewage sludge samples were dried at 60 °C for 72 h, finely ground (<0.5 mm), and stored in amber bottles at room temperature.

Sludge-Treated Soil. Topsoil samples (0−2.5 cm) from experimental sludge-treated fields were taken close to Zurich at two different locations: Wetzikon (7% organic carbon; 38% clay; 23% sand; 27% silt; pH 6.7) and Reckenholz (4% organic carbon; 18% clay; 54% sand; 21% silt; pH 6.9). Soil samples were collected using a steel cylinder 8 and 21 months after a sludge application rate of 25 t/ha. This corresponds to a 5 times the normal amount that is allowed in Switzerland every third year (e.g., 5 t/ha per 3 years). The soil samples were dried at 40 °C, passed through a 0.2-mm sieve, and stored in the dark at room temperature.[27]

Extraction, Cleanup, and Analysis. Dried samples of 200 mg of sewage sludge and 500 mg of sludge-treated soil were weighed, transferred into 11-mL stainless steel extraction cells from Dionex, and thoroughly mixed with ∼10 g of quartz sand. When spike and recovery experiments were performed, spiked samples were allowed to equilibrate overnight before extraction. A Dionex ASE 200 accelerated solvent extractor (Sunnyvale, CA) equipped with a solvent controller was used for extraction. Various aqueous mixtures were tested as extracting solvent, and a 50 mM aqueous phosphoric acid (pH 2.0) and acetonitrile mixture (1:1, v/v) was found to be optimal. The selected operating conditions were as follows: extraction temperature, 100 °C; extraction pressure, 100 bar; preheating period, 5 min; static extraction period, 15 min; final extraction volume ∼22 mL; solvent flush, 150% of the cell volume; nitrogen purge, 300 s; and number of extraction cycles, 4 and 6 for sewage sludge and sludge-treated soil samples, respectively. The ASE extracts were transferred to 200-mL volumetric amber flasks, filled with distilled water, and adjusted with 32% HCl to pH 3.0. The samples were then shaken and spiked with tosufloxacin surrogate standard (TOS-IS), yielding surrogate standard concentrations of 100 μg/L for sewage sludge and 50 μg/L for sludge-treated soil samples. In addition, the ion content of the diluted extracts was measured with a conductivity cell (Testo 240), for it could interfere with the cleanup.[19] Subsequently, a 10-mL aliquot of the diluted sludge or soil extracts was extracted by MPC disk cartridge and followed immediately by liquid chromatography fluorescence detection (LC-FLD) as described in ref 19.

Validation Parameters and Quality Control. To avoid photodegradation,[28] amber vials were used for preparing and storing standard solutions, as well as for the entire analytical procedure. Stability of FQs during sample pretreatment was checked by spiking a fresh sludge sample, which was subsequently allowed to dry at 60 °C for 96 h.[29] Extraction cells filled with quartz sand (inert matrix) were spiked with 0.5 μg of FQs, extracted at 100 °C, and analyzed using the same protocol,

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including sample cleanup, to confirm that no thermal degradation of FQs occurred during ASE extraction. TOS-IS was added as a surrogate standard to all samples to correct for losses during sample cleanup. Procedural blanks (quartz sand) were extracted for each set of 10 samples to control for laboratory contamination. An instrumental blank for the LC-FLD was run after every fourth sample to ensure no carryover during analysis. Duplicate ASE extractions were carried out for each sample. Breakthrough on MPC disk cartridges was investigated by extracting aliquots of 5, 10, 20, and 50 mL of the diluted sewage sludge extracts spiked with 100 μg/L TOS-IS in two stacked MPC disk cartridges. Multiple sequential extractions of the same sewage sludge (6 × 15 min) and sludge-treated soil (8 × 15 min) sample were conducted to ensure quantitative extraction. The overall method accuracy was determined by recovery studies of spiked sewage sludge and sludge-treated soil samples at different concentrations; duplicates of spiked sewage sludge at 2.5, 5, 10, and 20 mg/kg and spiked soils at 0.25, 0.5, 1, and 2 mg/kg were analyzed. The latter experiment was also used for standard addition studies. In such approach, the original concentration in the unspiked sample was obtained after extrapolating from the standard addition calibration to "zero spiked concentration".[30] Standard addition quantitation was then compared to the concentration obtained by using an internal calibration to review method accuracy. The precision of the entire procedure for sewage sludge and sludge-treated soil samples was determined by extracting six replicates containing native FQs and spiked with a TOS-IS surrogate standard prior to cleanup. Limit of detection (LOD) was defined as 3 times the standard deviation ($n = 10$) of FQ measurements in pure solvents at 330 pg on column. Because the limit of quantification (LOQ) calculated as 10 times the standard deviation was below the linear range, LOQ was set as the second lowest linear point of the calibration curve.[19]

RESULTS AND DISCUSSION

Selection of Extraction Technique. Because most methods described for the extraction of the quinolone oxolinic acid from sediments are based on manual or mechanical shake,[20,21] preliminary experiments were focused on the use of ultrasonication at room temperature. After extracting a same sample for several hours (8 × 30 min), only very low extraction efficiencies were achieved. ASE was then chosen, because of the stronger extraction power conferred due to enhanced solvent temperature. In addition to the high extraction efficiency of ASE, its accelerated extraction and the high degree of automation allowed a significantly higher sample throughput.

Accelerated Solvent Extraction. To understand the way in which ASE operational parameters affect the extraction of FQs from environmental samples, individual variables were considered. At first, various extraction solvent mixtures were tested. Once the optimum solvent mixture was found, the impact of varying temperature, pressure, and extraction time was studied. Finally, other factors were investigated regarding sample preparation prior to ASE, such as sample prewetting, swelling agent, and sample size. The development of the extraction procedure was performed with native digested sewage sludge and with sludge-treated soil samples. The obtained results presented as FQ concentration in sample are shown in Tables 1−4.

Table 1. ASE Efficiency of FQ from Sewage Sludge Using Various Aqueous Mixtures[a]

	concn[b] (mg/kg of dm)	
extraction solvent	ciprofloxacin	norfloxacin
pH = 2		
50 mM orthophosporic acid/acetonitrile (1:1)	**2.44 ± 0.18**	**2.36 ± 0.10**
50 mM orthophosporic acid/methanol (1:1)	2.31 ± 0.01	2.21 ± 0.04
50 mM orthophosporic acid/2-propanol (1:1)	2.01 ± 0.12	2.22 ± 0.11
pH = 7		
water/acetonitrile (1:1)	1.69 ± 0.12	1.60 ± 0.01
water/acetonitrile (1:3)	1.14 ± 0.10	1.28 ± 0.05
water/acetonitrile (3:1)	1.27 ± 0.06	1.18 ± 0.11
water/methanol (1:1)	1.63 ± 0.06	1.59 ± 0.07
water/2-propanol (1:1)	1.53 ± 0.04	1.52 ± 0.07
pH = 11		
0.1 M NaOH/acetonitrile (1:1)	2.19 ± 0.09	2.30 ± 0.08

[a] Selected operating conditions in boldface type. [b] Mean and standard deviation of duplicate determinations. ASE operating conditions: 100 °C, 100 bar, 4 cycles of 15 min.

Extraction Solvent. Table 1 shows the results of different aqueous mixtures in combination with an organic modifier (acetonitrile, methanol, or 2-propanol) regarding the extraction efficiency of FQs from sewage sludge. Pure organic solvents were not tested, since they were shown to be inefficient for extracting the quinolone oxolinic acid from sediments.[4] Several authors have already taken advantage of the increased solubility effects of hot liquid water (subcritical water) to extract organic pollutants from solid environmental samples,[31−33] and its use as an ASE solvent has recently been reported.[24,25] Among the organic modifiers studied in this study, acetonitrile showed better results than methanol or 2-propanol. Different water/acetonitrile mixtures (1:3, 1:1, 3:1) were studied. The extraction efficiency varied slightly as a function of the aqueous/organic modifier ratio, with the highest recoveries obtained at a ratio of 1:1.

Because of the zwitterionic character of FQs ($pK_{aCOOH} = 5.9-6.3$, $pK_{a\ NH_2} = 7.9-10.2$),[34] the effect of pH on the extraction efficiency of FQs was studied (Table 1). Under elevated temperature, strong acids (e.g., hydrochloric or nitric acid) oxidized the steel components of the extraction cell[29,35] so that the use of such acids had to be avoided. Thus, a dilute phosphoric acid (50 mM, pH 2.0) solution was selected as acidic extraction solvent and a NaOH (0.1M, pH 11.0) solution as a basic extraction solvent. The change in pH improved the results with maximum recoveries achieved at an acid pH. Because at low pHs the anionic sites of FQs and sewage sludge are protonated, it could be possible that the electrostatic repulsion between FQs and the sewage sludge surface might partly account for the better extraction efficiencies

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Table 2. Effects of Temperature, Pressure, and Time on the ASE of FQs from Sewage Sludge[a]

	concentrations[b] (mg/kg of dm)				
	temperature (100 bar, 4 × 15 min)				
compound	50 °C	75 °C	**100 °C**	125 °C	150 °C
ciprofloxacin	1.49 ± 0.01	2.25 ± 0.23	**2.52 ± 0.04**	2.58 ± 0.07	2.61 ± 0.15
norfloxacin	1.46 ± 0.17	2.13 ± 0.14	**2.40 ± 0.17**	2.45 ± 0.10	2.53 ± 0.09
	pressure (100 °C, 4 × 15 min)				
	50 bar	75 bar	**100 bar**	125 bar	150 bar
ciprofloxacin	2.34 ± 0.12	2.45 ± 0.09	**2.51 ± 0.17**	2.42 ± 0.09	2.41 ± 0.09
norfloxacin	2.47 ± 0.04	2.47 ± 0.06	**2.50 ± 0.12**	2.45 ± 0.10	2.32 ± 0.25

	time (100 °C, 100 bar)					
	20 min (4 × 5 min)	40 min (4 × 10 min)	40 min (2 × 20 min)	50 min (2 × 25 min)	**60 min (4 × 15 min)**	80 min (4 × 20 min)
ciprofloxacin	2.23 ± 0.05	2.44 ± 0.14	2.10 ± 0.04	2.30 ± 0.13	**2.45 ± 0.17**	2.47 ± 0.13
norfloxacin	2.06 ± 0.08	2.27 ± 0.02	1.91 ± 0.18	2.10 ± 0.20	**2.35 ± 0.13**	2.38 ± 0.21

[a] Selected operating conditions in boldface type. [b] Mean concentration and standard deviation of duplicate determinations.

at acidic pHs. Moreover, the enhanced extraction efficiency observed at high and low pHs could also be due to the increased aqueous solubility of FQs at extreme pHs, reaching a minimum at neutral pHs (zwitterionic form).[36] On the other hand, the fact that hot phosphoric acid has been reported to enhance the extraction of humic acids from soils[37] could also explain the better extraction of FQs, if FQs would be associated with the organic carbon fraction of the sample. On the basis of the presented results (Table 1), a 50 mM aqueous phosphoric acid/acetonitrile mixture (1:1) was selected as extraction solvent.

ASE Operating Parameters. Besides the extraction solvent, three operating parameters govern the extraction efficiency of ASE: temperature, pressure, and extraction time. For subcritical water extraction, however, efficiency seems to depend primarily on the extraction temperature and time, and pressure has only a minor influence.[31] The effect of temperature, pressure, and time on the extraction efficiency of FQs from sewage sludge is presented in Table 2.

(i) *Temperature* is expected to have a pronounced effect on the performance of ASE due to the importance of mass-transfer kinetics and solubility. We studied the effect of temperature on extraction efficiency of FQs by varying the system oven from 50 to 150 °C at increments of 25 °C in the different experiments. At higher extraction temperatures, it was observed that increasingly darker extracts were obtained, indicating a larger extraction of soluble organic solid matter. From 50 to 100 °C, the extraction efficiency of FQs was influenced by temperature, most probably due to an increase in extraction kinetics. In contrast, between 100 and 150 °C, the extraction efficiency remained constant, which indicates no influence of temperature on the equilibrium partitioning ($\Delta H \sim 0$). So a working temperature of 100 °C was selected to avoid oxidation of the extraction cell that may occur when phosphoric acid was used at higher temperatures.[29,35]

(ii) The effect of *pressure* on extraction efficiency of FQs was investigated at pressures ranging from 50 to 150 bar. Pressure is used to keep the extraction solvents liquid when the solvents are heated above their boiling points. Within the selected ranges, pressure changes showed no significant effects on the extraction efficiency of FQs (Table 2), and a working pressure of 100 bar was considered to be appropriate.

(iii) To investigate the effects of *extraction time* on extraction efficiency, time was varied from 20 to 80 min by prolonging the static time (from 5 to 20 min) and by augmenting the number of extraction cycles (from two to four cycles). In the static method, the long exposure to solvent allows the matrix to swell, thus improving the penetration of solvent into the sample interstices and the contact of the solvent with the analyte. On the other hand, by splitting the extraction time from one to more cycles, the introduction of fresh solvent maintains a favorable solvent/sample equilibrium and, hence, improving partitioning into the liquid phase. In our study, we observed a raise in extraction efficiency of FQs when the static time was increased from 5 to 10 min, but no substantial improvement was detected when it was prolonged to 15 or 20 min. Additionally, by increasing the continuous exposure to fresh solvent from 2 to 4 times, the partitioning of the FQs into the mobile phase was enhanced and thus also the extraction efficiency. Although a 10-min extraction performed four times with partial solvent exchange offered optimal extraction efficiency, 4 × 15 min was selected as operating conditions to ensure quantitative extraction even for matrixes that are difficult to extract.

To evaluate the ability of the method to quantitatively extract FQs from the studied matrixes, multiple sequential extractions of a sample were performed. This approach assumes that the final extraction removes all of the native analytes and that no additional analytes are associated with the sample by stronger interactions than the analytes that were already extracted.[38] For the same sample, up to 6 extraction cycles of 15 min for sewage sludge and 8 × 15 min for sludge-treated soils were separately collected and analyzed. The data from this series of experiments are shown in Figure 1. Defining the overall recovery of native FQs after

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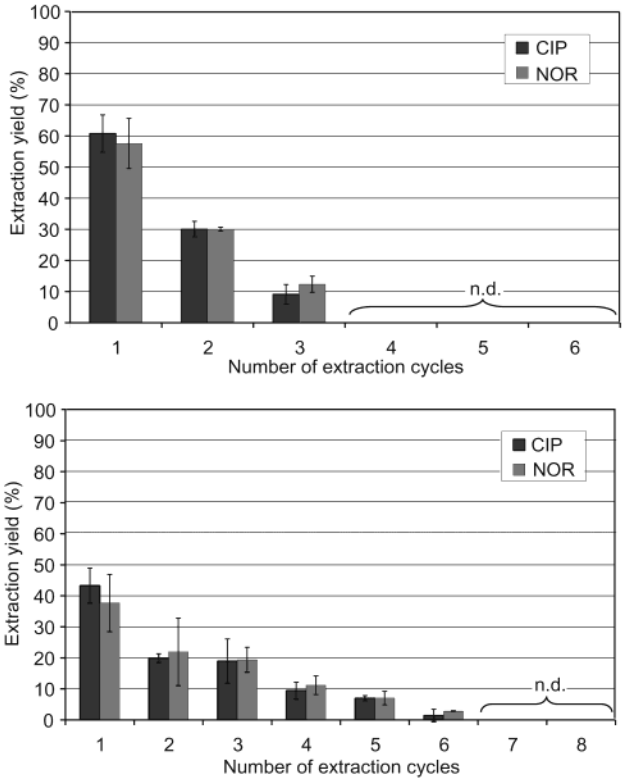

Figure 1. Extraction yields of FQs after multiple sequential extractions of 15 min each with 50 mM phosphoric acid/acetonitrile (1:1) at 100 °C and 100 bar. n.d., not detectable.

multiple sequential extractions as 100%, the recovery for the sewage sludge sample in the first extract varied from 52 to 65%, with an additional 28−32 and 7−14% in the second and third extracts, respectively. For sludge-treated soils, between 31 and 47% of native FQ was recovered in the first extract followed by an additional 14−30% in the second extract, 14−24% in the third, 8−13% in the fourth, 6−9% in the fifth, and 0−3% in the sixth extract. To ensure a quantitative extraction, the number of static extraction cycles was set to 4 × 15 min for sewage sludge and to 6 × 15 min for sludge-treated soils. The fact that FQs associated with sewage sludge are easier extractable than those from sludge-treated soil could indicate that the extraction efficiency depends on the type of matrix. On the other hand, these results could also be attributable to sample aging, so that stronger interactions between FQs and active sites in the sludge-treated soil samples (sludge disposal October 1999) would be expected compared to sewage sludges samples (sampling October 2000).

Pre-Extraction Sample Preparation. Among other experimental parameters that could affect ASE, we considered sample preparation as the most relevant. To allow a greater exposure surface and hence better diffusion of the extraction solvent into the matrix interstices, a thorough mixture between the sample and the quartz sand in the cell was required. In some studies from the literature, the effect of prewetting the matrix[39] or electrolyte addition[40] resulted in better extraction yields, especially when native residues were extracted. In both cases, the long exposure to solvent allowed the matrix to swell, thus improving the penetration of solvent into its interstices and increasing analyte recovery. Moreover, the addition of high concentrations of sodium ions in the second study[40] was reported to allow an interlayer swelling of the clay structure of the sediments. In our case, we investigated the influence of sample prewetting and the presence of swelling agent, by allowing sewage sludge and sludge-treated soils to swell for at least 15 h either with water or with aqueous solutions containing different amounts of NaCl (pH 5.5). The obtained results are presented in Table 3. As can be seen, sample prewetting or the presence of a swelling agent did not give higher efficiencies in either sewage sludge or soil extractions. On the contrary, the higher the salt content the lower the extraction efficiency, which might be attributed to a higher ionic activity in water due to the presence of $[Cl]^-$ ions.[36] Such an approach was therefore not further investigated and the samples were directly ASE-extracted, reducing overall duration of sample preparation.

Table 4 shows the dependence of extraction efficiency on sample size under optimal ASE conditions. Sample size was varied from 50 to 500 mg for sewage sludge and from 200 to 2000 mg for sludge-treated soil samples. Regardless of sample size, extraction efficiency was essentially identical up to 300 mg of sewage sludge and 1000 mg of soil. On the contrary, extraction yields decreased significantly for larger sample sizes, especially for 500 mg of sewage sludge and 2000 mg of soils, suggesting that higher extraction solvent volumes are required when larger sample amounts are extracted. The selected working sample size was 200 mg for sewage sludge and 500 mg for sludge-treated soil, to allow sufficiently low limits of quantification and a reliable quantitative analysis.

Postextraction Sample Preparation and Cleanup. Sample preparation after extraction is considered an integral part of an extraction procedure, in which a simplified procedure is preferred. Nonetheless, to achieve a selective method for FQs, a cleanup step was required. The postextraction sample preparation was optimized by employing the solid-phase extraction already described for FQs in wastewater.[19] MPC disk cartridges were already shown to be the most selective for FQs and to provide the best recovery rates,[19] presumably because of a more specific interaction between the nonpolar and ionic sites of FQs and the sorbent. The fact that in the present study an organic modifier was used as extraction solvent in the ASE appeared to be a major problem for cleanup with MPC disk cartridges, for which aqueous samples are preferable. To overcome this, the ∼22-mL ASE extract was diluted to 200 mL with water, so that the organic content was reduced to ∼5% acetonitrile/95% water. Out of the diluted extract, a 10-mL aliquot was then solid-phase extracted with MPC disk cartridges. This dilution approach was favored over a complete evaporation of the acetonitrile fraction, which would need more sample handling, lead to longer postextraction sample pretreatment, and include the uncertainty of FQ losses during sample evaporation because of possible sorption to glass.

Method Validation and Quality Control. The optimized ASE−SPE LC-FLD method was validated for both sewage sludge and sludge-treated soil (selected conditions summarized in the Experimental Section) with the most important parameters presented in Table 5.

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Table 3. Effect of Prewetting and the Presence of a Swelling Agent on the ASE of FQs from Sewage Sludge and Sludge-Treated Soil Samples[a]

prewetting swelling agent	concentrations[b] (mg/kg of dm)			
	sewage sludge[c]		sludge-treated soil[d]	
	ciprofloxacin	norfloxacin	ciprofloxacin	norfloxacin
none	**2.27 ± 0.09**	**2.19 ± 0.04**	**0.22 ± 0.01**	**0.27 ± 0.03**
water	2.00 ± 0.20	1.94 ± 0.03	0.21 ± 0.01	0.26 ± 0.03
NaCl 0.1 M	2.21 ± 0.21	2.21 ± 0.15	0.21 ± 0.01	0.26 ± 0.02
NaCl 0.3 M	1.78 ± 0.20	1.76 ± 0.11	0.19 ± 0.01	0.23 ± 0.01

[a] Selected operating conditions in boldface type. [b] Mean concentration and standard deviation of duplicate determinations. [c] ASE operating conditions: 100 °C, 100 bar, four cycles of 15 min. [d] ASE operating conditions: 100 °C, 100 bar, six cycles of 15 min.

Table 4. Effect of Sample Size on ASE of FQs from Sewage Sludge and Sludge-Treated Soil Samples[a]

compound	concentration[b] (mg/kg of dm)				
	sewage sludge[c]				
	50 mg	100 mg	**200 mg**	300 mg	500 mg
ciprofloxacin	2.44 ± 0.08	2.33 ± 0.06	**2.42 ± 0.06**	2.22 ± 0.21	1.86 ± 0.16
norfloxacin	2.45 ± 0.10	2.49 ± 0.06	**2.37 ± 0.07**	2.35 ± 0.11	1.79 ± 0.15
	sludge-treated soil[d]				
	200 mg	**500 mg**	750 mg	1000 mg	2000 mg
ciprofloxacin	0.23 ± 0.01	**0.21 ± 0.02**	0.19 ± 0.01	0.18 ± 0.01	0.11 ± 0.01
norfloxacin	0.26 ± 0.01	**0.25 ± 0.01**	0.26 ± 0.01	0.22 ± 0.01	0.14 ± 0.01

[a] Selected operating conditions in boldface type. [b] Mean concentration and standard deviation of duplicate determinations. [c] ASE operating conditions: 100 °C, 100 bar, four cycles of 15 min. [d] ASE operating conditions: 100 °C, 100 bar, six cycles of 15 min.

Table 5. Quality Control Parameters

parameter	sewage sludge[a]		sludge-treated soil[b]	
	ciprofloxacin	norfloxacin	ciprofloxacin	norfloxacin
recovery[c] ($n = 8$), %	89 ± 4	88 ± 4	80 ± 6	84 ± 5
precision[d] ($n = 6$), %	10	8	8	11
limit of detection, mg/kg of dm	0.12		0.05	
limit of quantification, mg/kg of dm	0.45		0.18	
linear range, mg/kg of dm	0.45−75		0.18−30	

[a] ASE operating conditions: 100 °C, 100 bar, four cycles of 15 min. [b] ASE operating conditions: 100 °C, 100 bar, sic cycles of 15 min. [c] Mean recoveries and standard deviation of *n* replicates of spiked sewage sludge and spiked sludge-treated soil sample. [d] Given as relative standard deviation of *n* replicates.

Thermal Degradation Studies. Because ASE extractions were performed at elevated temperatures, thermal degradation was of potential concern. Overall average recoveries obtained for spiked FQs on clean quartz sand were 99 ± 3% for CIP and 95 ± 3% for NOR, giving no evidence of thermal degradation.

Breakthrough Experiments. FQs were quantitatively isolated from aliquots of 5 and 10 mL of diluted ASE extracts of sewage sludge by the first of two stacked MPC disk cartridges. Occasionally, residues of the FQ (<5% of the amount found in the first disk cartridge) were detected in the second cartridge, which could be derived from the remaining ∼ 5% acetonitrile content in the diluted extract. When aliquots of 20 and 50 mL of diluted ASE extracts were enriched, breakthrough occurred on the order of 10 and 20%, respectively. Finally, an aliquot of 10 mL of the diluted extracts was used for sample cleanup. Because the high ion content in the sample could induce earlier breakthrough and thus reduce extraction efficiencies of FQs, conductivity of diluted extracts was controlled.[19] Conductivity of diluted extracts following pH adjustment was always less than 1.5 mS/cm; thus, method validation was only applicable for samples not exceeding this value.

Accuracy and Precision. Spiked analytes are not exposed to the same matrix active sites as the native pollutants. Therefore, recoveries obtained with spiked samples might be different from those with native samples. In our case, however, multiple sequential extraction from sewage sludge and sludge-treated soils confirmed that a quantitative extraction was achieved for native FQs (see Figure 1); therefore, we adopted such spiking experiments to evaluate the overall method recoveries, including sample cleanup. Overall method recoveries ($n = 8$) ranged from 82 to 94% for sewage sludge ($m_{CIP} = 89 \pm 4\%$, $m_{NOR} = 88 \pm 4\%$, $m_{TOS-IS} = 93 \pm 5\%$) and from 75 to 92% for sludge-treated soil samples ($m_{CIP} = 80 \pm 6\%$, $m_{NOR} = 84 \pm 5\%$, $m_{TOS-IS} = 87 \pm 8\%$). Such results are comparable to those obtained for wastewater ($m_{CIP} = 92 \pm 5\%$, $m_{NOR} = 91 \pm 10\%$, $m_{TOS-IS} = 100 \pm 5\%$)[19] and surface

Table 6. Concentrations of Ciprofloxacin and Norfloxacin in Sewage Sludge from Different Wastewater Treatment Plants and Sludge-Treated Soil Samples from Different Experimental Fields to Which Sewage Sludge Had Been Applied at a Rate of 25 t/ha

		concentration[a] (mg/kg of dm)	
sample type	sampling site	ciprofloxacin	norfloxacin
untreated raw sludge[b]	Dübendorf	1.40 ± 0.12	1.54 ± 0.03
	Zurich-Werdhölzli	2.03 ± 0.20	1.96 ± 0.15
digested sludge[b]	Kloten-Opfikon	2.42 ± 0.06	2.37 ± 0.07
	Zurich-Werdhölzli	2.27 ± 0.20	2.13 ± 0.19
sludge-treated soil[c]	Wetzikon	0.35 ± 0.04	0.32 ± 0.01
(8 months after application)	Reckenholz	0.40 ± 0.03	0.29 ± 0.01
sludge-treated soil[c]	Wetzikon	0.28 ± 0.01	0.27 ± 0.01
(21 months after application)	Reckenholz	0.27 ± 0.04	0.30 ± 0.01

[a] Mean concentration and standard deviation of duplicate determinations. [b] ASE conditions: 100 °C, 100 bar, 4 cycles of 15 min. [c] ASE conditions: 100 °C, 100 bar, 6 cycles of 15 min.

water (m_{CIP} = 85 ± 10%, m_{NOR} = 87 ± 9%, m_{TOS-IS} = 97 ± 6%);[15] thus, the FQ losses were associated with the cleanup procedure more than with the SE extraction. Spiking experiments at different concentrations were also used for standard addition curves, with the aim to ensure the accuracy of the quantified concentrations in native sewage sludge and sludge-treated soils samples. Relative standard variation between internal standard and standard addition quantification varied between 4 and 6% for values above the LOQ and up to 15% for concentrations below the LOQ. Relative standard deviations (n = 6) in the range of 8−11% were achieved for extraction, cleanup, and analysis of six replicates of sewage sludge and sludge-treated soils containing native FQs.

LOD/LOQ and Linearity. Limits of detection and quantification for 200 mg of sewage sludge and for 500 mg of soil samples are presented in Table 5. The linear range of FQ analysis was defined from the LOQ (second lower linear point) until 50 ng on column, corresponding to 75 mg/kg of dry matter (dm) for sewage sludge and 30 mg/kg of dm for soil samples.

Determination of FQs in Environmental Samples. Several environmental samples were analyzed in order to assess the applicability and performance of the developed method. The two FQs occurring in the Swiss aquatic environment CIP and NOR were quantified in several sewage sludges taken from municipal treatment plants in the region of Zurich (Table 6). Concentrations of FQs in untreated raw sewage were between 1.40 and 2.03 mg/kg of dm sewage sludge, being in the same range as those found in digested sludge (2.13−2.42 mg/kg of dm). The relatively high levels in sewage sludge indicate the high affinity of FQs toward solids and favored sorption to sewage sludge during wastewater treatment.

The FQs CIP and NOR were also determined in two sludge-treated soil samples from experimental fields near Zurich (Table 6 and Figure 2). The samples represent two different soils with different composition (see the Experimental Section) and treated with sludge at a rate of 25 t/ha. Note that the sludge application ratio was 5 times the allowed amount in Switzerland every third year (5 t/ha). Topsoil concentrations 8 months after sludge application ranged from 0.29 to 0.40 mg of FQ/kg of dm and, after 21 months, from 0.27 to 0.30 mg of FQ/kg of dm. Such concentrations are within the range of those found for veterinary pharmaceuticals in soils after liquid manure dispersion (0.04−0.20 mg/kg tetracycline).[1] Despite no observed significant reduction in FQ concentrations in topsoil layers in either of the two

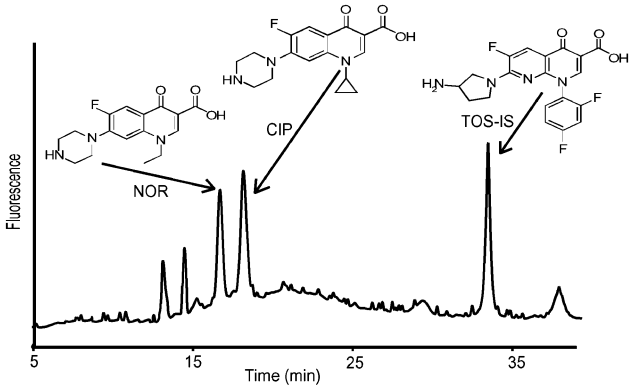

Figure 2. LC-FLD chromatogram of a topsoil sample (0−2.5 cm) collected 21 months after sludge application from the experimental field at the Reckenholz area. Determined concentrations: 0.27 and 0.30 mg/kg of dm for ciprofloxacin and norfloxacin, respectively.

soils in the 13-month study period, partial FQ degradation could have occurred previous to the first sampling campaign (8 months after sludge application). Interestingly, two additional peaks appear in the more polar fraction of the sludge-treated soil chromatogram, which could well indicate some FQ degradation products (generally more polar than the parent compound). In any case, the results presented here demonstrate that trace amounts of FQs persist (and may well accumulate) in the terrestrial environment after sludge application.

CONCLUSIONS

This work presents a new and highly selective ASE−SPE procedure for the determination of FQs in solid environmental samples. Its applicability for extracting FQs from sewage sludge and solid matrixes at the microgram per kilogram level is demonstrated, and the suitability of the previously reported LC-FLD method to solid matrix extracts is proven. The ASE efficiency particularly depended on the composition and pH of the solvent and the temperature and extraction time selected.

The presented results confirmed the hypothesis that FQs become highly enriched in sewage sludge. Additionally, this study demonstrated that FQs reach the terrestrial environment via the disposal of sewage sludge to agricultural soils. Moreover, the persistence of trace amounts of FQs in sludge-treated soils up to several months after application was demonstrated.

The developed method can be a valuable tool for investigating the fate and behavior of FQs during wastewater treatment by performing mass balance studies through the analysis of sewage sludge and, moreover, to further assess the occurrence, persistence, and transport of FQs in sludge-treated soils. These approaches are currently being addressed in our laboratory, confirming the usefulness of this analytical method for process-oriented field studies.[41] Since both FQs studied showed identical extraction behavior, besides ciprofloxacin and norfloxacin the described method should also be applicable to other human- or veterinary-use FQs (e.g., sarafloxacin, enrofloxacin). Furthermore, applications to other solid environmental matrixes such as sediments should be possible without major modifications.

(41) Golet, E. M. Ph.D. Thesis ETH, Zurich, No. 14690, 2002.

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